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Comments: Re: Application No. 10/603,501 by Franano

Dear Examiner Afremova,

As requested. I am transmitting a copy of the Declaration by F. Nicholas Franano, MD, dated May 17, 2005, that was submitted under 37 C.F.R. §1.132 during prosecution of Application No. 09/669,051.

Please let me know if I can be of any further assistance.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	FRANANO	Confirmation No.:	2612
Serial No.:	09/669,051	Art Unit:	1651
Filed:	September 24, 2000	Examiner:	Srivastava, Kailash C
For:	METHODS FOR TREATING AN ARTERY OR VEIN IN A HUMAN SUBJECT (as amended)	Attorney Docket No.:	31110-0002

DECLARATION UNDER 37 C.F.R. § 1.132 OF F. NICHOLAS FRANANO, M.D.

The Director
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

1. I, F. Nicholas Franano, M.D., hereby declare as follows:

2. I am the President and Chief Executive Officer of Proteon Therapeutics LLC, 4420 Madison Avenue, Suite 180, Kansas City, MO 64111 ("Proteon Therapeutics"), assignee of U.S. Application Serial No. 09/669,051, filed September 24, 2000 (the "'051 application") and the inventor of the technology disclosed therein. I also am an Assistant Professor of Radiology (Part-Time, Visiting) at Johns Hopkins University in Baltimore, Maryland. A copy of my Curriculum Vitae is attached as Exhibit 1.

3. I am familiar with the disclosure and data presented in the '051 application. It is my opinion that, in September, 2000, one of ordinary skill in the art, armed with

the teachings of the '051 application and publicly available knowledge, could successfully practice the methods of the present invention in accordance with the specification of the '051 application without undue experimentation. The foregoing opinion is based on the reasons and facts set forth below.

The Animal Models Employed in the Studies Described Herein Are Recognized as Valid Predictors of Outcomes in Humans

4. Those of ordinary skill in the art of cardiovascular biology understand that it is often unethical or impractical to perform routine and preliminary medical experiments in human subjects. Thus, the field has developed a variety of animal models in which such studies may be performed, and in which experimental therapies may be optimized before being applied in human patients. Examples of such art-recognized animal models of the human vascular system include the rabbit artery and vein, *see e.g.* Strauss BH *et al.*, Extracellular Matrix Remodeling After Balloon Angioplasty Injury in a Rabbit Model of Restenosis, Circulation Research 1994;75:650-658 (Exhibit 2), Wilensky RL *et al.*, Vascular Injury, Repair, and Restenosis After Percutaneous Transluminal Angioplasty in the Atherosclerotic Rabbit, Circulation 1995;92:2995-3005 (Exhibit 3) and references cited therein, and the porcine artery and vein, *see, e.g.*, Johnson MS *et al.*, The Porcine Hemodialysis Access Model, Journal of Vascular and Interventional Radiology 2001;12:969-977 (Exhibit 4). Previously, these models have been used to develop a host of new medical, pharmacological or surgical treatments for use in humans, including new treatments to prevent vascular restenosis and new surgical procedures to permit preclinical evaluation of interventional devices and techniques intended to reduce the effects of intimal hyperplasia in human patients undergoing dialysis. The rabbit and pig models are widely accepted in the art of vascular biology as predictive of success for treatment of humans and have successfully been used to evaluate and introduce new human vascular therapies.

Effects of Elastase Administration on the Diameter And Elastin Content of Arteries and Veins

5. Using rabbit and porcine animal models, my associates at Proteon Therapeutics and I have demonstrated that local administration of elastase to the wall of an artery or vein produces long-lasting dilation of the treated artery or vein. The vasodilation produced by elastase treatment was associated with a marked increase in the diameter of the treated vessel and of its lumen, and with a dramatic decrease in elastin fiber content of the vessel wall.

Dose Titration of Elastase and Determination of the Effects of Elastase Administration on the Luminal Diameter of the Rabbit Carotid Artery

6. In an initial dose titration study, the left carotid artery of rabbits was surgically exposed and 3mL of a solution containing various concentrations of porcine pancreatic elastase was locally administered to the external surface. The treated artery was then examined 28 days after treatment. As shown in Table 1, local administration of porcine elastase at concentrations of at least 20 units per milliliter (U/mL) produced statistically significant dilation of the arterial lumen. At 10 U/mL, there was dilation in 3/4 animals at 28 days. At 20 U/mL, there was dilation in 4/4 animals at 28 days. At 40 U/mL, there was dilation in 4/4 animals at 28 days. At 200 U/mL, there was dilation in 8/8 animals at 28 days. Moreover, there was no evidence of aneurysmal dilation or vessel rupture, even after administrations of elastase at concentrations that were ten times greater than those required to see a statistically significant increase in lumen diameter (200 U/ml). In control studies, angiography was performed on animals 42 days after the left carotid artery was treated with saline using a similar technique; dilation was observed in 0/4 animals. These studies demonstrate that an effective dose of elastase may readily be determined by one of ordinary skill in the art, using the information provided in the specification of the '051 application in combination with the teachings of the prior art, and that administration of elastase to the rabbit carotid artery produces a significant increase in luminal diameter.

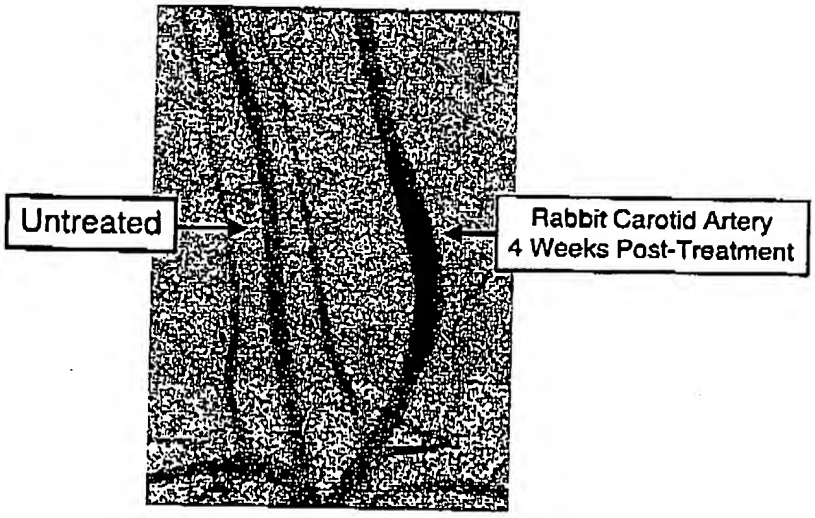
Table 1. Administration of Elastase Increases the Luminal Diameter of the Rabbit Carotid Artery

Concentration	Lumen Diameter (mm)
No treatment	2.2 ± 0.18
10 U/mL	2.5 ± 0.18
20 U/mL	2.9 ± 0.19
40 U/mL	3.2 ± 0.13
200 U/mL	3.2 ± 0.26

Angiographic Demonstration That Administration of Elastase Increases the Luminal Diameter of the Rabbit Carotid Artery

7. The dilation produced by administration of elastase to the carotid artery of the rabbit was easily visible by angiography. Figure 1 shows an angiographic image of the carotid arteries of a rabbit 28 days after treatment of the left artery (on the right in the angiograph) by administration of 3 mL of a solution containing 40 U/mL of porcine elastase. The pronounced dilation produced by the procedure is easily observed by comparing the left carotid artery with the right carotid artery, which serves as an untreated contralateral control. These results demonstrate that administration of elastase to the rabbit carotid artery dramatically increases luminal diameter.

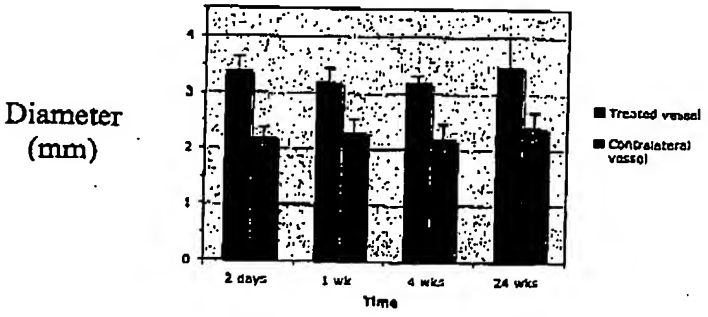
Figure 1. Angiographic Image of Rabbit Carotid Artery after Administration of Porcine Elastase



The Vasodilation of Rabbit Carotid Arteries Produced by Local Administration of Elastase is Stable Over Time

8. The dilation of the rabbit carotid artery produced by elastase treatment was stable over time. Lumen diameter was measured by angiography at 2 days, 1 week, 4 weeks, and 24 weeks after treatment with 40 U/mL of porcine pancreatic elastase for 15 min. As shown in Figure 2, the resulting dilation was stable. After the initial dilation, the treated vessels showed no subsequent statistically significant change in lumen diameter.

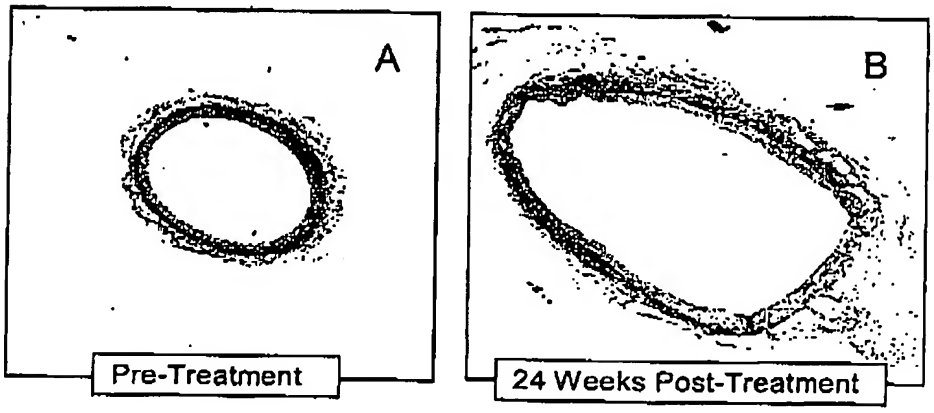
Figure 2. Administration of Elastase Results In A Stable Increase in the Luminal Diameter of Treated Rabbit Carotid Arteries



Histological Analyses Show That Local Administration of Elastase Dramatically Increases Both the Diameter of the Lumen and the Diameter of the Treated Arteries Themselves

9. Tissue sections of treated and control artery segments in the experiments described above were examined histochemically to determine the effects of elastase treatment on the diameter of the vessel and on the diameter of the lumen (Figure 3) and elastin distribution (Figure 4) in the arterial wall. In these studies, vessel segments were pressure-fixed *in situ* using formalin at 100 mm Hg. The fixed arteries were then embedded in paraffin, sectioned, and stained either with trichrome stain for determination of luminal diameter (Figure 3) or with Verhoeff-Van Gieson stain for determination of elastin content of the arterial wall (Figure 4). As shown in Panels A and B of Figure 3, which were obtained under identical magnification, elastase treatment dramatically increased both the luminal diameter of the rabbit carotid artery and the diameter of the vessel itself at 24 weeks after treatment.

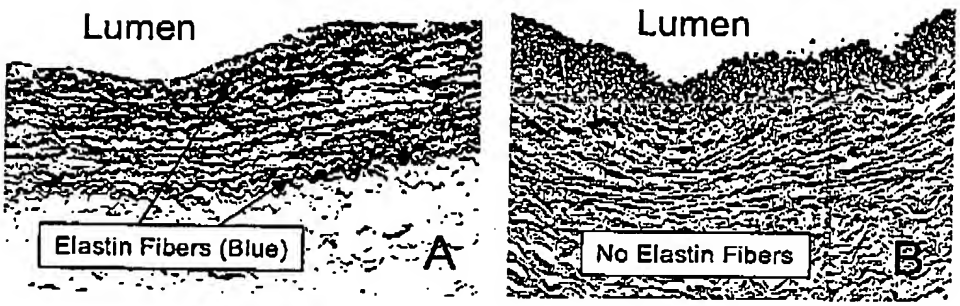
Figure 3. Histological Observation Confirms That Administration of Elastase to Rabbit Carotid Arteries Dramatically Increases the Diameter of the Lumen and the Diameter of the Treated Vessels



Histological Analyses Show That Administration of Elastase Dramatically Reduces Elastin Content of the Vessel Wall

10. The histochemical examination shown in Panels A and B of Figure 4 demonstrates that elastase treatment eliminates positively-stained elastin fibers from the vessel wall. These results show that administration of elastase results in proteolysis of elastin in the vessel wall, consistent with the proposition that the arterial vasodilation observed after administration of elastase to the carotid artery is due to reduced elastin content of the vessel wall.

Figure 4. Elastin Content of Rabbit Carotid Artery Wall After Administration of Porcine Elastase



Changes in Arterial Wall Thickness Alone Cannot Account for the Vasodilatory Effects of Elastase Administration

11. Histological studies also were performed on untreated rabbit carotid arteries to determine their mean thickness before treatment. In these studies, the rabbit left common carotid artery was surgically exposed and pressure fixed with 5% formalin at approximately 120 mm Hg for 15 minutes. The fixed vessel segments were excised and embedded in paraffin. Slides were cut and stained with H&E. Wall thickness was measured at four equidistant positions around each vessel cross-section. As shown in Table 2, the average wall thickness was determined to be 58.9 $\pm$ 25.3 microns (n = 4 animals; error is $\pm$ 1 SD). Because administration of elastase results in the dilation of vessels from 2.2 mm (pre-treatment) to 3.2 mm (post-treatment) in diameter (Table 1), an increase of 1.0 mm or

approximately 17 times the thickness of untreated vessel, the dilation produced by elastase treatment cannot be due to a decrease in vessel wall thickness alone. These studies confirm that elastase treatment increases not only the diameter of the lumen of the treated vessel but also increases the diameter of the treated vessel itself.

Table 2. Wall Thickness of Untreated Rabbit Carotid Arteries

Vessel	Mean Thickness (microns)	Std Dev (microns)
Left Common Carotid Artery	58.9	25.3

Qualitatively Similar Dilation of Rabbit Carotid Arteries and Depletion of Vessel Wall Elastin Content Was Obtained By Administration of Recombinant Human Pancreatic Elastase

12. To demonstrate dilation using elastases from other species, we performed an additional set of experiments on the rabbit carotid artery using recombinant human pancreatic elastase (specifically, human pancreatic elastase I), for which the gene had been cloned and sequenced. *See* U.S. Patent No. 5,162,205 (Takiguichi *et al.*, issued 11/10/1992). In these studies, 6 mL of a solution containing 8.5 Units/mL of recombinant human pancreatic elastase was locally administered to the exposed surface of the rabbit common carotid artery for 30 minutes. At two days after treatment, angiography was performed to determine vessel diameter and histological analyses were performed to determine the elastin content of the vessel wall. For the histological studies, the vessels were pressure-fixed *in situ* using formalin at 120 mm Hg. The fixed arteries were then embedded in paraffin, sectioned, and stained with Verhoeff-Van Gieson stain for determination of elastin content of the arterial wall. The vasodilation produced by administration of recombinant human elastase was observed by comparing the treated carotid artery with its untreated contralateral control (Figure 5). As shown in Figure 6, the vessel treated with recombinant human elastase (panel B) showed a complete absence of elastin fibers, while abundant elastin was seen in the wall

of an untreated artery (Panel A). These studies demonstrate that the vasodilation and elastin depletion caused by elastase administration is obtained using elastase from more than one mammalian species.

Figure 5. Angiographic Image of Rabbit Carotid Artery after Administration of Recombinant Human Elastase

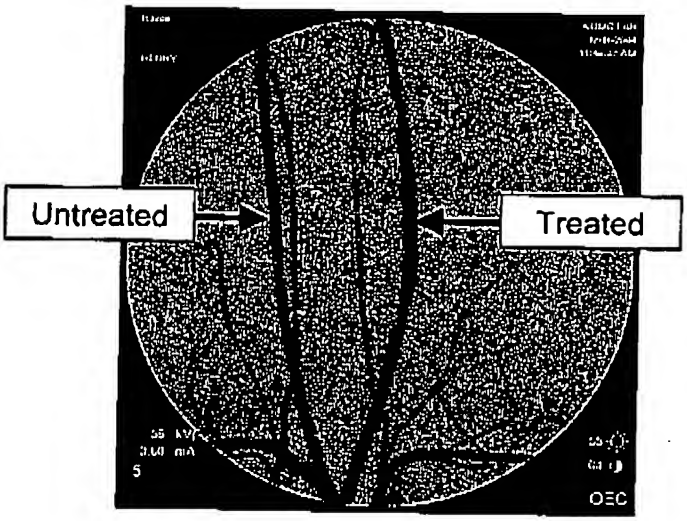
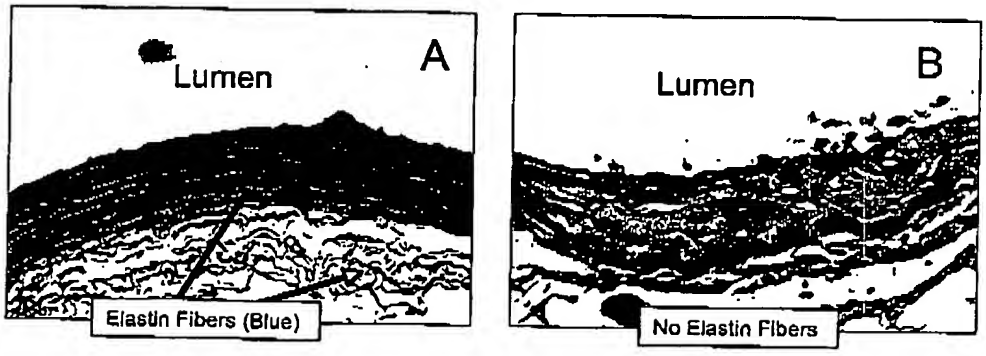


Figure 6. Elastin Content of Rabbit Carotid Artery Wall After Administration of Recombinant Human Elastase

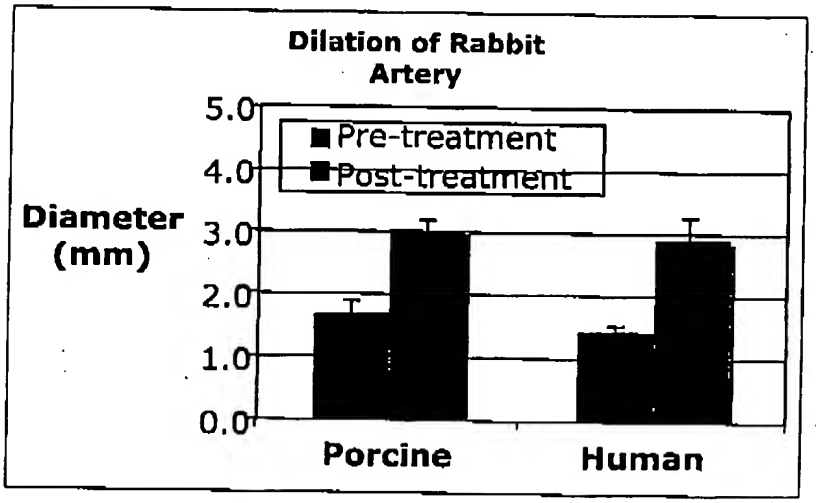


Direct Comparison of the Effects of Porcine and Recombinant Human Elastase on the Rabbit Carotid Artery

13. To compare the *in vivo* efficacy of porcine and recombinant human elastases, rabbit common carotid arteries were exposed and treated topically with

recombinant human pancreatic elastase I (3 mL of a 20 U/mL solution) for 15 minutes (n=5). Images of the vessels were taken before, during, and immediately after treatment using a digital camera. Average vessel diameter in treated regions was calculated with the assistance of ImagePro Plus software. The results obtained from these studies then were compared to data obtained from previous studies using the same dose of porcine pancreatic elastase. The results are shown in Figure 7. Error bars represent ± 1 SD. Using a t-test for unpaired samples, both porcine and recombinant human elastase showed statistically significant dilation of the diameter of the treated vessel. There were no statistically significant differences between the results obtained with the porcine and human enzymes. Thus, both recombinant human elastase and porcine elastase caused arterial dilation of equal magnitude.

Figure 7. Comparison of Recombinant Human and Porcine Elastase on the Dilation of the Rabbit Carotid Artery



Determination of the Effects of Elastase Administration on the Luminal Diameter of the Rabbit Jugular Vein

14. Additional studies were performed to confirm that elastase administration also produced dilation of veins. In these studies, the rabbit jugular vein was surgically exposed and topically treated with 3 mL of a solution containing porcine elastase

(40 U/ml) or the vasodilator papavarine (6 mg/ml) as a control. Treated vessels were examined either with venography (Figure 8) or histology (Figure 10) at 28 days after treatment. As shown in Figure 8, elastase treatment resulted in an average increase in lumen diameter of approximately 1.5 mm. Control treatment had no statistically significant effect on vessel size at 28 days. Representative venograms are shown in Figure 9. Panel A shows a vein segment before treatment. Panel B shows the same vein segment after external administration of 40 U/mL of porcine elastase. Elastase treatment also completely eliminated positively-stained elastin fibers from the vessel wall (Figure 10), as previously demonstrated in rabbit arteries. These studies demonstrate that treatment with elastase causes dilation of veins.

Figure 8. Administration of Porcine Elastase Increases the Luminal Diameter of the Rabbit Jugular Vein

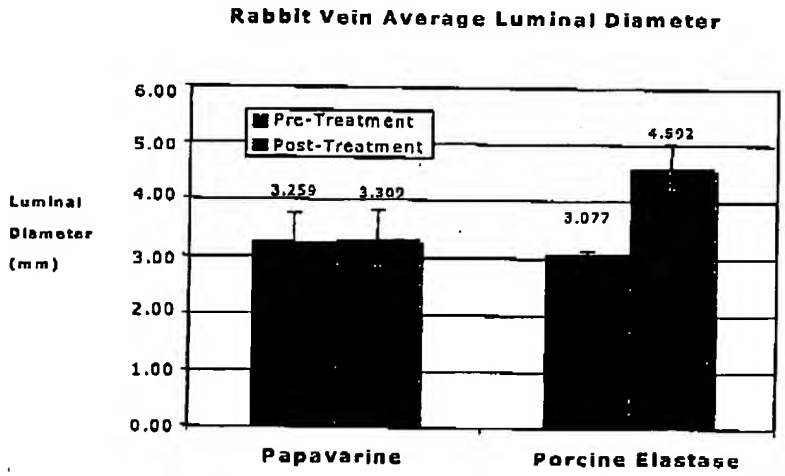


Figure 9. Venographic Demonstration of the Effects of Administration of Porcine Elastase on the Rabbit Jugular Vein

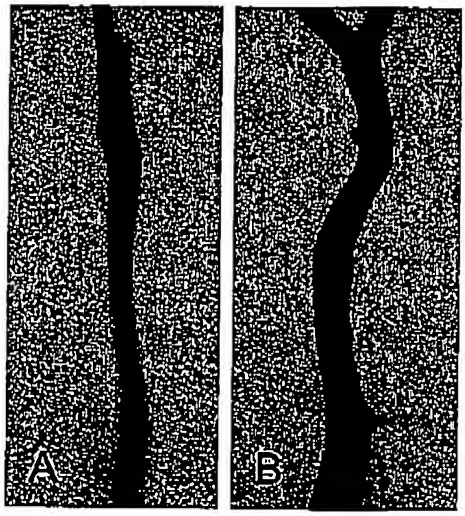
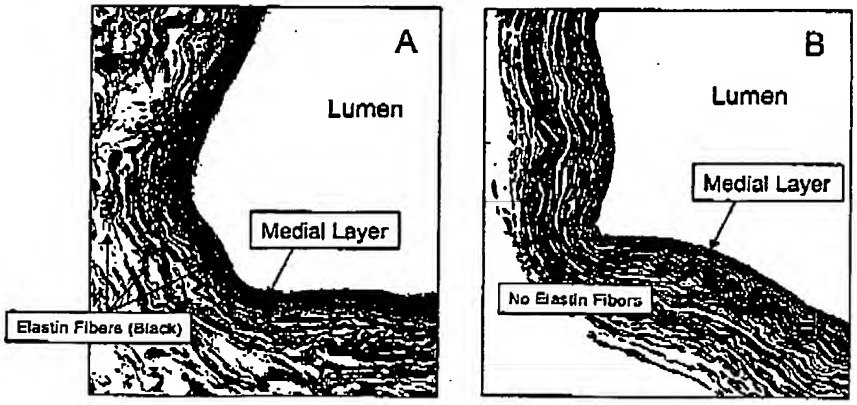


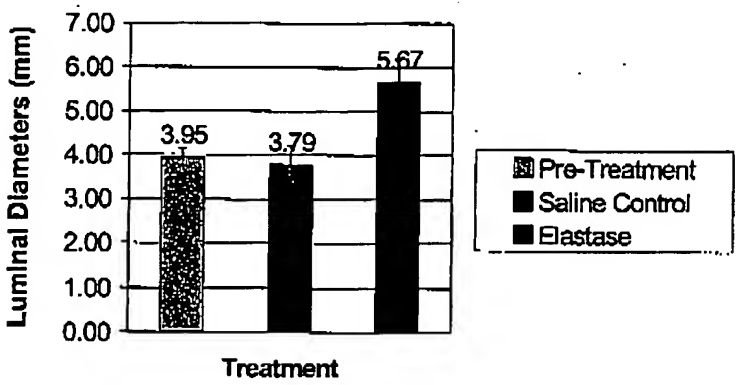
Figure 10. Elastin Content of Rabbit Carotid Artery Wall After Administration of Porcine Elastase



Elastase-mediated Vasodilation by Catheter-mediated Administration of Elastase

15. We next confirmed that similar levels of vasodilation were achieved by administering the elastase solution through a catheter. In these studies, performed in the porcine model, an Infiltrator catheter was introduced into the left common carotid artery of the pig, and advanced through the circulatory system to the left femoral artery. The balloon was inflated for two minutes and 0.5 ml of a solution containing 320 U/ml of porcine pancreatic elastase was injected through microinjector nozzles mounted on the surface of the balloon and into the vessel wall over a period of about thirty seconds. The balloon was deflated, removed, flushed, and reinserted into the right femoral artery of the same animal. A similar procedure then was repeated except that a control saline solution was injected instead of the elastase solution. Two days after treatment, follow up angiography was performed to quantify the luminal diameter. Dilation was seen in 2/3 arteries treated with elastase and in 0/3 arteries treated with the saline control. As shown in Figure 11, the dilated arteries showed an approximately 43% increase in luminal diameter. Saline treatment in the same animal resulted in a small but statistically insignificant reduction in luminal diameter.

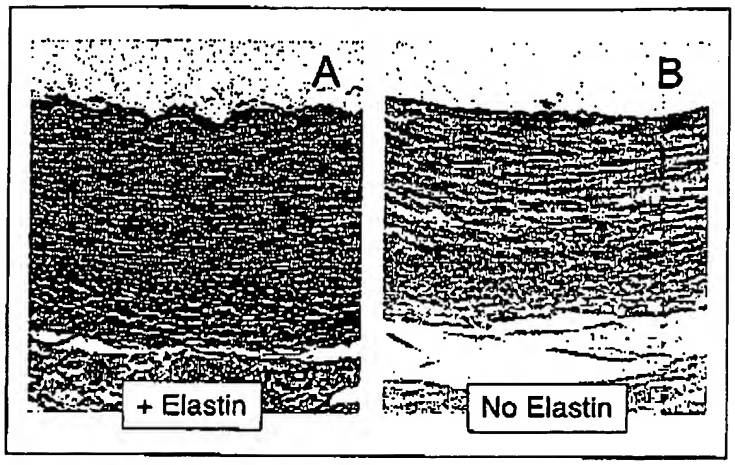
Figure 11. Catheter-mediated Administration of Elastase Causes Vasodilation of the Porcine Femoral Artery



Histological Analyses Show That Catheter-mediated Administration of Elastase Dramatically Reduces Elastin Content of the Vessel Wall

16. Tissue sections of treated and control artery segments in the pig experiments described above were examined histochemically to determine the effects of porcine elastase treatment on elastin distribution (Figure 12). In these studies, vessel segments were pressure-fixed *in situ* using formalin at 100 mm Hg. The fixed arteries were then embedded in paraffin, sectioned, and stained with Verhoeff-Van Gieson stain for determination of elastin content of the arterial wall. As shown in Panels A (saline control) and B (elastase treatment), catheter-mediated elastase treatment eliminated positively-stained elastin fibers from the vessel wall.

Figure 12. Elastin Content of Porcine Femoral Artery Wall
After Catheter-mediated Administration of Elastase



**Catheter-mediated Elastase Treatment
to Dilate Veins in the Porcine Model**

17. Further studies were performed in which elastase was administered by catheter to porcine veins. In these studies, a venogram was performed on the right jugular vein of a pig via an ear vein. An Endobionics Microsyringe catheter then was introduced into the right femoral vein of the pig, and advanced through the circulatory system to the right jugular vein. The balloon was inflated and 2.5 ml of a solution containing 100 U/ml of porcine elastase was injected through a microneedle mounted on the surface of the balloon and into the vessel wall over a period of about 120 seconds. The balloon was deflated and removed, and a second venogram was performed 80 minutes after completion of the injection. The results of these studies, shown in Figure 13, demonstrate that elastase administration produces a noticeable increase in the luminal diameter of the jugular vein. The resulting dilation was stable over time (Figure 14). This result demonstrates dilation of veins by catheter-mediated administration.

Figure 13. Venographic Demonstration of the Effects of Catheter-mediated Administration of Elastase on the Porcine Jugular Vein

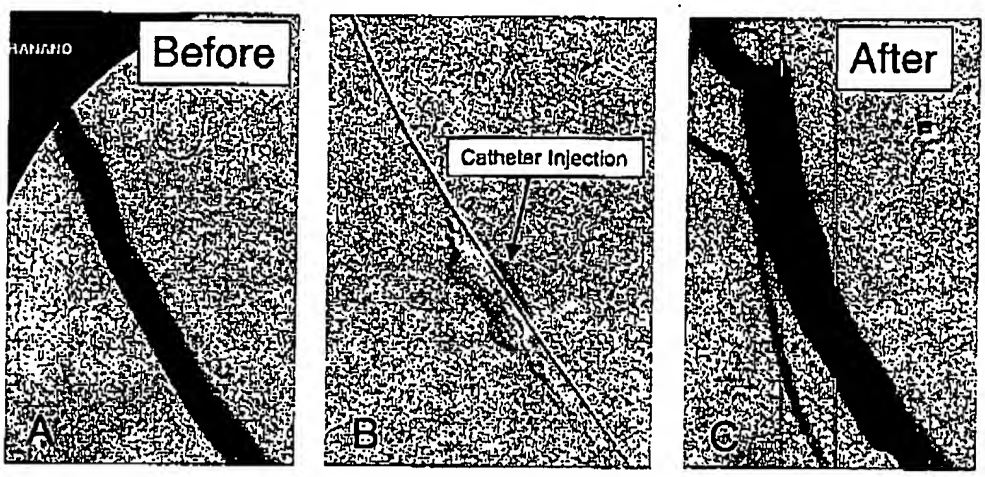
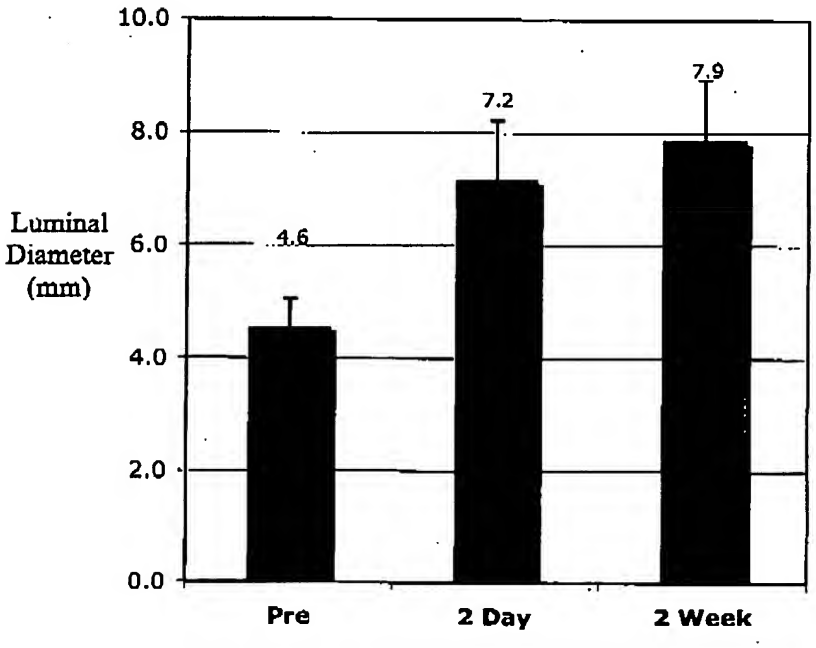


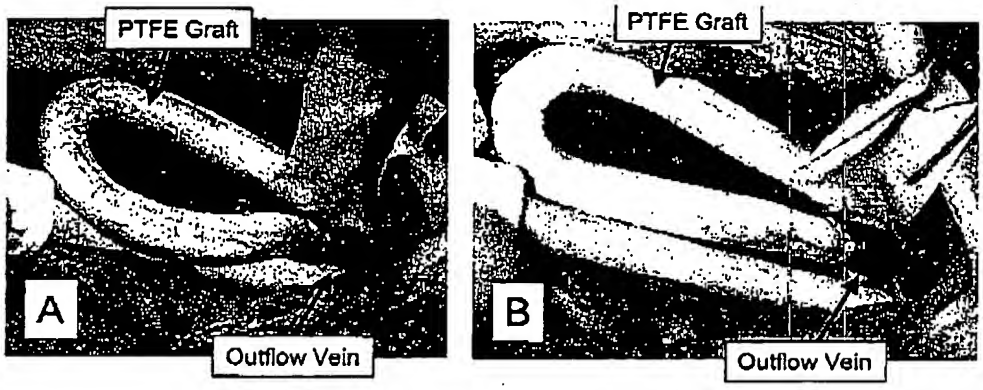
Figure 14. The Increase in the Luminal Diameter of the Rabbit Jugular Vein Produced by Catheter-mediated Administration of Elastase is Stable Over Time



Elastase Administration to Dilate Veins in Hemodialysis Access Grafts

18. An additional series of experiments was performed to confirm the utility and efficacy of elastase treatment for the dilation of veins or arteries to be used in an arteriovenous hemodialysis graft. In these studies, the femoral artery and vein of a Yorkshire pig was exposed and a 4 mm diameter polytetrafluoroethylene (PTFE) bridge graft was constructed between the artery and vein. After flow was established in the graft, the outflow vein was treated with 20 U/mL of porcine pancreatic elastase for 15 minutes. Images before (A) and after (B) treatment are shown in Figure 15 below. Elastase treatment noticeably increased the diameter of the treated vein.

Figure 15. Elastase Administration Causes Dilation in an Arteriovenous Hemodialysis Graft Model



Conclusions

19. As shown by these studies and data, it is my opinion that one of ordinary skill in the art could successfully practice the methods of the present invention in accordance with the specification and without undue experimentation. When practiced in accordance with the specification, the claimed methods increase the diameters of elastase-treated vessels and of their lumens. Moreover, a ten fold

excess of elastase over the minimal effective dose did not lead to rupture, aneurysmal dilatation, or any apparent local or systemic toxicity.

20. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: May 17, 2005

F. Nicholas Franano, M.D.

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